

WHAT IS CLAIMED IS:

1. A method of flowing fluid in a non-sipper microfluidic device, the method comprising: flowing fluid through the non-sipper microfluidic device to emulate a fluid flow profile in a microfluidic device comprising an external capillary, wherein the fluid flow profile results from flowing one or more sample from an external source into a microfluidic device.

2. The method of claim 1, wherein the non-sipper microfluidic device comprises a planar microfluidic device.

3. The method of claim 1, wherein the external source comprises a microwell plate.

4. The method of claim 1, wherein flowing fluid through the non-sipper microfluidic device comprises creating one or more sample plug and one or more buffer plug in the non-sipper microfluidic device, which one or more sample plug and one or more buffer plug emulate fluid flow from the external source into the microfluidic device via the external capillary.

5. The method of claim 4, wherein creating the one or more sample plug and the one or more buffer plug comprises:

- (i) loading a sample from a first source into a channel of the non-sipper microfluidic device,
- (ii) loading a buffer from a second source into the channel;
- (iii) applying pressure to the sample in the channel, thereby creating the one or more sample plug and transporting the one or more sample plug through the channel; and,
- (iv) applying pressure to the buffer in the channel, thereby creating the one or more buffer plug and transporting the one or more buffer plug through the channel.

6. The method of claim 5, comprising alternately performing step (i) and step (ii).

7. The method of claim 5, comprising repeating steps (i) and (ii).

5 8. The method of claim 5, comprising continuously performing step (iii) and step (iv).

9. The method of claim 5, comprising alternately performing step (i) and step (ii) while simultaneously performing step (iii) and step (iv).

10 10. The method of claim 5, step (iii) and step (iv) comprising simultaneously applying a first pressure to the sample and a second pressure to the buffer, wherein the first pressure and the second pressure are different.

11 11. The method of claim 5, wherein the first source and the second source comprise internal reservoirs.

12 12. The method of claim 5, comprising loading the sample from the first source into the channel of the non-sipper microfluidic device by applying a first electrokinetic gradient between the first source and a waste reservoir and loading the buffer from the second source into the channel by applying a second electrokinetic gradient between the second source and the waste reservoir.

13 13. The method of claim 12, wherein the waste reservoir comprises an internal reservoir.

14 14. The method of claim 12, comprising alternately applying the first electrokinetic gradient and the second electrokinetic gradient.

15 15. The method of claim 14, comprising alternately applying the first electrokinetic gradient and the second electrokinetic gradient and simultaneously applying pressure to the sample in the channel and to the buffer in the channel.

16. The method of claim 5, comprising loading the sample from the first source into the channel by applying pressure to the sample and loading the buffer from the second source into the channel by applying pressure to the buffer.

17. The method of claim 16, comprising alternately applying pressure to the sample and to the buffer.

18. The method of claim 17, comprising alternately applying pressure to the sample in the first source and to the buffer in the second source and concurrently applying pressure to the sample in the channel and to the buffer in the channel.

19. The method of claim 1, wherein flowing fluid through the non-sipper microfluidic device comprises

- (i) flowing a sample from a first internal source into a non-sipper main channel via a capillary emulator channel;
- (ii) flowing the sample through the non-sipper main channel; and,
- (iii) flowing one or more reagent from at least a second internal source into the non-sipper main channel via a non-sipper side channel.

20. The method of claim 19, wherein the capillary emulator channel simulates the external capillary.

21. The method of claim 19, wherein the non-sipper main channel simulates a sipper main channel.

22. The method of claim 19, wherein the non-sipper side channel simulates a sipper side channel.

23. The method of claim 20, claim 21, or claim 22, wherein simulates comprises having substantially the same hydrodynamic resistance as an equivalent channel in the microfluidic device comprising the external capillary.

24. The method of claim 20, claim 21, or claim 22, wherein simulates comprises having substantially the same length, width, and depth as an equivalent channel in the microfluidic device comprising the external capillary.

5 25. The method of claim 20, claim 21, or claim 22, wherein simulates comprises flowing substantially the same amount of the one or more reagent or the sample as an equivalent channel in the microfluidic device comprising the external capillary.

10 26. An assay development device, which assay development device emulates a microfluidic sipper device, the assay development device comprising:

(i) a non-sipper microfluidic substrate comprising a plurality of microscale channels, the plurality of microscale channels comprising:

(a) a main channel; and,

(b) at least one capillary emulator fluidly coupled to the main channel;

15 and,

(ii) at least a first fluid control element fluidly coupled to the main channel in the non-sipper microfluidic device.

20 27. The assay development device of claim 26, wherein the non-sipper microfluidic substrate comprises a planar microfluidic substrate.

28. The assay development device of claim 26, which main channel emulates a sipper device main channel.

25 29. The assay development device of claim 28, wherein the main channel comprises a first hydrodynamic resistance, a length, a width, a depth, or a flow characteristic, which hydrodynamic resistance, length, width, depth, or flow characteristic is substantially equal to the sipper device main channel.

30 30. The assay development device of claim 26, wherein the capillary emulator comprises a microscale channel, which microscale channel comprises a hydrodynamic resistance, a length, a width, a depth, or a flow characteristic, which

hydrodynamic resistance, length, width, depth, or flow characteristic is substantially equal to a sipper capillary in the microfluidic sipper device.

31. The assay development device of claim 26, further comprising an electrokinetic controller fluidly coupled to the capillary emulator, which capillary emulator comprises:

a waste reservoir, which waste reservoir is fluidly coupled to the main channel;

a sample well fluidly coupled to the waste reservoir and to the main channel;

a buffer well fluidly coupled to the waste reservoir and to the main channel;

wherein the first fluid control element applies a pressure differential between the waste reservoir and the pressure source, and the electrokinetic controller alternately applies an electrokinetic gradient between the sample well and the waste reservoir and between the buffer well and the waste reservoir.

32. The assay development device of claim 26, wherein the capillary emulator comprises:

a sample well fluidly coupled to the main channel; and,

a buffer well fluidly coupled to the main channel;

wherein the first fluid control element

applies a first pressure to a sample in the sample well, thereby flowing the sample into the main channel;

applies a second pressure to a buffer in the buffer well, thereby flowing the buffer into the main channel; and,

applies a third pressure to the sample in the main channel or to the buffer in the main channel.

33. The assay development device of claim 32, wherein the fluid control element alternates between applying the first pressure and applying the second pressure and concurrently applies the third pressure.

34. The assay development device of claim 26, the first fluid control element comprising a pressure source or an electrokinetic controller.

35. An assay development device, the device comprising:

(i) a non-sipper microfluidic device comprising a plurality of microscale channels, the plurality of microscale channels comprising:

5 (a) a main channel;

(b) a first reagent well fluidly coupled to the main channel;

(c) a second reagent well fluidly coupled to the main channel; and,

(d) a waste reservoir fluidly coupled to the main channel, the first reagent well, and the second reagent well; and,

10 (ii) a fluid control system, which fluid control system comprises:

(a) a pressure source fluidly coupled to the main channel; and,

(b) an electrokinetic controller operably coupled to the main channel;

wherein the fluid control system applies a pressure differential between the waste reservoir and the pressure source, and alternately applies an electrokinetic gradient

15 between the first reagent well and the waste reservoir and between the second reagent well and the waste reservoir.

36. A method of fabricating an assay development device, the method comprising:

20 (i) providing a non-sipper microfluidic substrate; and,

(ii) fabricating two or more channels within the non-sipper microfluidic substrate, the two or more channels emulating an external capillary and a main channel of a microfluidic sipper device.

37. The method of claim 36, the non-sipper microfluidic substrate comprising a planar substrate.

38. The method of claim 36, wherein emulating the external capillary and the main channel of the microfluidic sipper device comprises having one or more of:

30 substantially the same hydrodynamic resistance, substantially the same width, substantially the same depth, substantially the same length, or substantially the same flow characteristics as the external capillary or the main channel of the microfluidic sipper device.

39. The method of claim 36, the two or more channels comprising two or more of: a capillary emulator, a main channel, a side channel, or a reservoir.

5 40. The method of claim 38, wherein having substantially the same flow characteristics as the external capillary or the main channel in the microfluidic sipper device comprises providing substantially the same amount of fluid flow in substantially the same amount of time.